EMSP
Environmental Management Science Program
Project Summary Fact Sheet • October 1999

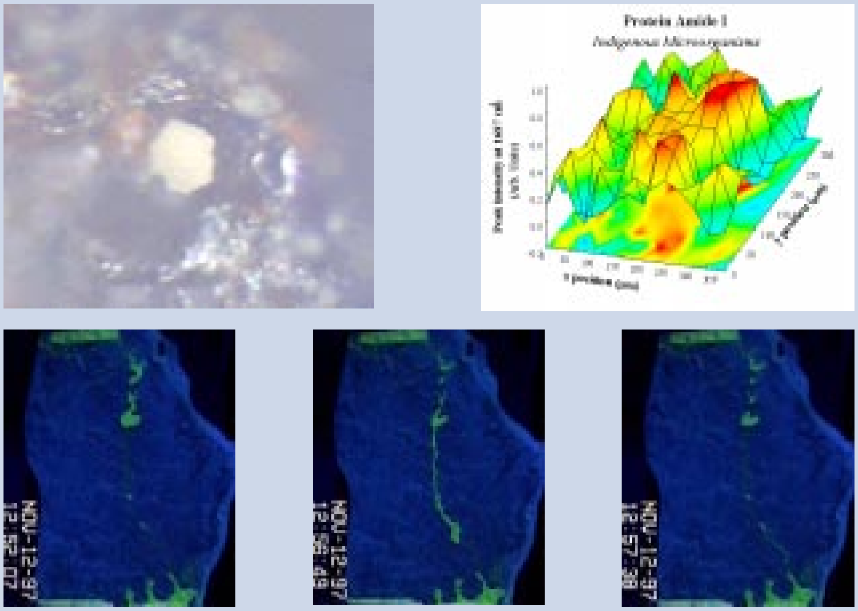
ADVANCED BIOREMEDIATION AND ENHANCED NATURAL ATTENUATION

BETTER UNDERSTANDING OF MICROBIAL/PLANT PHYSIOLOGY/ENVIRONMENTAL INTERACTIONS IS NECESSARY FOR IMPROVED AND MORE EXTENSIVE USE OF BIOREMEDIATION AND PHYTOREMEDIATION TECHNOLOGIES

The projects in this category are concerned with developing a better understanding of microbial/plant physiology/environmental interactions in order to yield improved bioremediation technologies.

These efforts include:

- developing a fundamental understanding of the interplay between fluid flow and microbial transformations within the fractured rock vadose zone;
- providing an improved understanding and predictive capability of the mechanisms that allow metal-reducing bacteria to be effective in bioremediation;
- developing innovative methods of investigating the mechanism and extent of in-situ bioremediation of chlorinated organic solvents;
- developing an improved understanding of microbial physiology as it relates to destruction of organic contaminants; and
- developing an improved understanding of plant physiology as it relates to destruction or removal of organic and heavy metal contamination.



Biological Activity in a Fractured Rock Vadose Zone
In investigations conducted by a Lawrence Berkeley National Laboratory team (210), a reflectance microscope photo of a basalt rock chip inoculated with intrinsic microbes (top left), reveals the presence of an attached colony, seen as the cream-colored shape. Mapping of the region (top right) shows biomarker peaks at locations corresponding to the bacteria observed in the photo. The figures below show images taken several minutes apart of water seepage (green) through a basalt fracture inoculated with intrinsic microbes. Over the 20-day experiment, changes in water seepage patterns corresponded to an increase in bacteria numbers and a decrease in the surface tension of the water.

PROBLEMS/SOLUTIONS

- An effective, inexpensive method for remediating groundwater contamination is needed. Advanced biological treatment methods, including in-situ bioremediation and phytoremediation, may provide such methods.
- New methods are needed to meet the challenge of remediating many U.S. Department of Energy (DOE) sites with subsurface contaminants that are difficult to access because of their depth and complex geology.
- Developing an improved understanding of the relationship between physical and microbial heterogeneity, a bacterial response to environmental stresses, and the mechanisms controlling the partitioning of bacteria between aqueous and solid phases will improve the design and implementation of in-situ bioremediation.
- Developing an improved understanding of the mechanisms by which plants extract contaminants from the soil and transport them to various parts of the plant is essential before phytoremediation can be extensively applied.
- Developing an improved understanding and predictive capability of the mechanisms that allow metal-reducing bacteria to be effective in the bioremediation of subsurface environments will contribute directly to planned remediation at Hanford and the Savannah River Site.

ANTICIPATED IMPACT

- The expanded use of bioremediation technologies will allow cost-effective cleanup of organic contamination of soil and groundwater at DOE sites, thereby eliminating the long-term source for groundwater contamination and reducing human health risk. In addition, significant costs from long-term treatment and compliance operations at DOE sites can be avoided.
- More than 600 billion gallons of contaminated groundwater and 50 million cubic meters of contaminated soil are targeted for remediation at DOE sites. Thus, improvements in remediation efficiencies can produce large savings in this program, which is expected to cost $6 billion.

Advanced Bioremediation

A Lawrence Berkeley National Laboratory (LBNL) team (210) is developing a fundamental understanding of the interplay between fluid flow and microbial transformations within the fractured rock vadose zone. The examination of fluid flow dynamics within the fracture plane and of biotransformation processes at the microscale has provided mechanistic insights regarding biological activity on mineral surfaces.

An Oak Ridge National Laboratory (ORNL) team (55013) is investigating the fundamental properties of biofiltration units. The vapor-phase volatile organic compounds (VOCs) are passed through a tube containing the microbes that can metabolize them, and this research team has studied means to control biofilm overgrowth, predictive models for the operation of these systems, isolation and identification of the microorganisms, and several other problems related to long-term use of effective biofiltration technology for removal of priority pollutants.

The ORNL and coworkers team (55267) is conducting research to provide an improved understanding and predictive capability of the mechanisms that allow metal-reducing bacteria to be effective in bioremediation of subsurface environments contaminated with toxic metals and radionuclides.

Isotopic ratio measurements have been used by the LBNL/Idaho National Engineering and Environmental Laboratory (INEEL) team (55351) to characterize two underground contamination sites at INEEL. Ratios of the isotopic abundances of the carbon isotopes suggested that some biodegradation of the less toxic organic compounds is occurring. Other isotopic ratios have been used to show that there is no exchange between two different aquifer layers and that the groundwater at one site has a significant contribution of evaporated water from a disposal pond.

The Argonne National Laboratory team is developing innovative methods for investigating the mechanism and the extent of in-situ bioremediation of chlorinated organic solvents. The methods use precise isotopic ratio measurements of chlorine and carbon in reactant and product species.

Microbial Science

The Stanford University team is (1) determining the biochemical pathways for microbial reductive dehalogenation of cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC); (2) determining the chemical requirements, especially the type and quantity of electron donors needed by the process; and (3) evaluating kinetics of the process.

The University of California – Davis and coworkers team (54681) is investigating the attachment/detachment mechanisms used by growing anaerobic microorganisms (which are capable of reductive dechlorination) and will incorporate this information into a mechanistic-based predictive model for heterogeneous porous media.

***Thlaspi goesingense* Growing in Its Native Ultramafic Habitat Near Redschlag/Austria**
The Northern Arizona University team is investigating, at the molecular level, the role of histidine biosynthesis in hyperaccumulation in *Thlaspi goesingense*.

The Pacific Northwest National Laboratory team has undertaken a genetic analysis of stress responses in soil bacteria to better understand the response of both microbial community and individual bacteria to the environmental stresses encountered at contaminated sites. Examples of stresses that were examined include low nutrients, low oxygen, and mixed pollutants.

The INEEL and coworkers team (55416) is determining the distribution of biologically active zones of contaminant degradation and its relationship to vertical heterogeneity in a fractured geologic medium.

The ORNL/University of Georgia team (55033) is designed to gain understanding of the molecular and catalytic properties of enzymes that have been chemically modified to be soluble and catalytically active in organic solvents. A poly(ethylene glycol)-modified enzyme was found to have an 11-fold higher catalytic efficiency for degradation of pentachlorophenol in 15% acetonitrile than did the native enzyme in pure aqueous buffer. The catalytic behaviors of chemically modified, hyperthermophilic metalloenzymes (ferredoxin, hydrogenase, and aldehyde oxidoreductase) were also examined in organic solvents. It is expected that these studies will ultimately lead to development of high-performance enzymatic catalysts for use in organic solvents.

The Michigan State University/University of Illinois team is isolating and characterizing microorganisms that use chlorinated ethenes (perchloroethylene or PCE, trichloroethylene or TCE, cDCE, and VC) as electron acceptors in their energy metabolism. They have isolated four methanogenic enrichment cultures that dechlorinated PCE to ethene and two cultures that dechlorinated 1,2-dichloropropane to propene.

The University of Maryland – Baltimore team is developing and testing molecular methods that will allow rapid characterization of microbial communities in contaminated systems. The research is developing a large sequence database of microbial genomic DNA sequences.

The University of Texas – Austin/University of Pittsburgh team is investigating bacteria that have peptides or antibodies on the surface and are capable of binding toxic metal species; these bacteria will be used to make bioadsorbents for groundwater remediation.

Another LBNL and coworkers team (55264) is undertaking an integrated physical (geophysical and hydrologic) and microbial study using innovative geophysical imaging and microbial characterization methods to identify key scales of physical heterogeneities that affect the biodynamics of natural subsurface environments. This research will improve understanding of the relationship between subsurface physical and microbial heterogeneity, which will aid design of bioremediation strategies.

A fourth LBNL team (55343) is using protein engineering to convert existing enzymes (catalytic antibodies) that will catalyze the hydrolysis of halogenated aromatics. Progress includes initial development of general selections and screens for identifying novel catalysts from libraries of antibody mutants.

Plant Science

The University of Georgia team (54837) is using genetic engineering to incorporate single-gene traits into plants, enabling them to process heavy metals and remediate heavy metal contamination. They engineered a small model plant to use a highly modified bacterial mercuric ion reductase to detoxify ionic mercury, reducing it to much less toxic metallic mercury.

The University of Washington team is testing the ability of poplar trees to take up and transform chlorinated methanes, ethanes, and ethylenes into innocuous products such as carbon dioxide, water, and chloride. They are also determining the rate of uptake and transformation by poplar of TCE.

The Northern Arizona University team is isolating and characterizing the key genetic information needed for plants to exhibit the ability to naturally accumulate high levels of metals in their shoots (metal hyperaccumulation). They are investigating, at the molecular level, the role of histidine biosynthesis in hyperaccumulation in *Thlaspi goesingense*.

The University of California (UC) – San Diego team has cloned cDNA from wheat roots; the cDNA was then transferred into yeast cells, which show enhanced uptake of cadmium, lead, and calcium.

The Scripps Research Institute team is investigating the function of a novel heavy metal pump in a model plant system, *Arabidopsis*. The long-term goal of this type of research is to understand how heavy metals are transported across the plasma membrane of plant cells.

The UC – Davis/Riverside team (55118) is conducting research to improve understanding of the plant root/soil interfaces on metal mobilization and subsequent transport into the root and shoot. Greater understanding of the following areas is needed: root exudation for chelating and reducing metal ions; cell wall binding of metal ions; and root-microbial associations that influence metal ion availability.

The U.S. Department of Agriculture team is seeking to define genes of the fission yeast *Schizosaccharomyces pombe*, which are involved in heavy metal tolerance and sequestration. Two approaches are used: cloning and characterizing genes that complement cadmium hypersensitive mutants of fission yeast, and isolating genes that confer cadmium hypertolerance.

Project Teams

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